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MEMBRANE-BASED LATERAL FLOW ASSAY DEVICES THAT UTILIZE PHOSPHORESCENT DETECTION

BY

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Background of the Inv_ntion

Phosphorescence is the result of a three-stage process. In the first stage, energy is supplied by an external source, such as an incandescent lamp or a laser, and absorbed by the phosphorescent compound, creating excited electronic triplet states (as opposed to fluorescence, which only has a singlet excited state). In the second stage, the excited states exist for a finite time during which the phosphorescent compound undergoes conformational changes and is also subject to a multitude of possible interactions with its molecular environment. During this time, the energy of the excited states is partially dissipated, yielding relaxed states from which phosphorescence emission originates. The third stage is the phosphorescence emission stage wherein energy is emitted, returning the phosphorescence compound to its ground states. The emitted energy is lower than its excitation energy (light or laser) and thus of a longer wavelength. This shift or difference in energy or wavelength allows the emission energy to be detected and isolated from the excitation energy.

Various phosphorescent compounds, such as metalloporphyrins, have been proposed for use in immunoassays. Unfortunately, many of the proposed techniques fail to solve the problem of quenching. Specifically, oxygen and water are strong quenchers of triplet states and may cause decay of the phosphorescence signal, thereby limiting its use in most practical assay applications. In addition, many of the techniques that have been proposed are simply ill equipped for use in lateral flow, membrane-based devices. For example, in a lateral flow, membrane-based assay device, the concentration of the analyte is reduced because it is diluted by a liquid that may flow through the porous membrane. However, background interference becomes increasingly problematic at such low analyte concentrations because the phosphorescent intensity is relatively low. Because the structure of the membrane also tends to reflect the excited light, the ability of a detector to accurately measure the phosphorescent intensity of the labeled analyte is substantially reduced. In fact, the intensity of the emitted phosphorescence signal may be three to four orders of magnitude smaller than the excitation light reflected by the porous membrane. Many membranes,

such as nitrocellulose membranes, also exhibit strong fluorescence when excited in the UV and visible regions. This fluorescence can interfere with the accuracy of phosphorescence measurements.

As such, a need currently exists for a simple, inexpensive, and effective system for using phosphorescence as a detection technique for membrane-based, lateral flow assay devices.

Summary of the Invention

In accordance with one embodiment of the present invention, a method for detecting the presence or quantity of an analyte residing in a test sample is disclosed. The method comprises:

i) providing a lateral flow assay device that comprises a porous membrane in fluid communication with detection probes, the detection probes comprising a phosphorescent label encapsulated within a matrix, wherein the porous membrane defines a detection zone within which is immobilized a capture reagent that is configured to bind to the detection probes or complexes thereof;

ii) contacting the detection probes with the test sample;

iii) allowing the detection probes and the test sample to flow to the detection zone;

iv) exciting the phosphorescent label at the detection zone to generate an emitted detection signal; and

v) measuring the intensity of the detection signal, wherein the amount of the analyte within the test sample is proportional to the intensity of the detection signal.

In accordance with another embodiment of the present invention, a lateral flow assay device for detecting the presence or quantity of an analyte residing in a test sample is disclosed. The assay device comprises a porous membrane in fluid communication with detection probes. The detection probes comprise a phosphorescent metal complex encapsulated within a matrix. The porous membrane defines a detection zone within which a capture reagent is immobilized that is configured to bind to the detection probes or complexes thereof to generate a detection signal, wherein the amount of the analyte in the test sample is proportional to the intensity of the detection signal.

Other features and aspects of the present invention are discussed in greater detail below.

Brief Description of the Drawings

A full and enabling disclosure of the present invention, including the best mode thereof, directed to one of ordinary skill in the art, is set forth more particularly in the remainder of the specification, which makes reference to the appended figures in which:

Fig. 1 is a perspective view of one embodiment of a lateral flow, membrane-based device of the present invention;

Fig. 2 is a schematic diagram of one embodiment of a phosphorescence reader that may be used in the present invention, including representative electronic components thereof;

Fig. 3 is a schematic diagram of another embodiment of a phosphorescence reader that may be used in the present invention, including representative electronic components thereof; and

Fig. 4 is a schematic diagram of still another embodiment of a phosphorescence reader that may be used in the present invention, including representative electronic components thereof.

Repeat use of reference characters in the present specification and drawings is intended to represent same or analogous features or elements of the invention.

Detailed Description of Representative Embodiments

Definitions

As used herein, the term "analyte" generally refers to a substance to be detected. For instance, analytes may include antigenic substances, haptens, antibodies, and combinations thereof. Analytes include, but are not limited to, toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), drug intermediaries or byproducts, bacteria, virus particles and metabolites of or antibodies to any of the above substances. Specific examples of some analytes include ferritin; creatinine kinase MB (CK-MB); digoxin; phenytoin; phenobarbitol; carbamazepine; vancomycin; gentamycin; theophylline; valproic acid; quinidine; luteinizing hormone (LH); follicle stimulating hormone (FSH); estradiol, progesterone; C-reactive protein; lipocalins; IgE antibodies; cytokines; vitamin B2

micro-globulin; glycated hemoglobin (Gly. Hb); cortisol; digitoxin; N-acetylprocainamide (NAPA); procainamide; antibodies to rubella, such as rubella-IgG and rubella IgM; antibodies to toxoplasmosis, such as toxoplasmosis IgG (Toxo-IgG) and toxoplasmosis IgM (Toxo-IgM); testosterone; salicylates; acetaminophen; hepatitis B virus surface antigen (HBsAg); antibodies to hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM (Anti-HBC); human immune deficiency virus 1 and 2 (HIV 1 and 2); human T-cell leukemia virus 1 and 2 (HTLV); hepatitis B e antigen (HBeAg); antibodies to hepatitis B e antigen (Anti-HBe); influenza virus; thyroid stimulating hormone (TSH); thyroxine (T4); total triiodothyronine (Total T3); free triiodothyronine (Free T3); carcinoembryoic antigen (CEA); lipoproteins, cholesterol, and triglycerides; and alpha fetoprotein (AFP). Drugs of abuse and controlled substances include, but are not intended to be limited to, amphetamine; methamphetamine; barbiturates, such as amobarbital, secobarbital, pentobarbital, phenobarbital, and barbital; benzodiazepines, such as librium and valium; cannabinoids, such as hashish and marijuana; cocaine; fentanyl; LSD; methaqualone; opiates, such as heroin, morphine, codeine, hydromorphone, hydrocodone, methadone, oxycodone, oxymorphone and opium; phencyclidine; and propoxyhene. Other potential analytes may be described in U.S. Patent Nos. 6,436,651 to Everhart, et al. and 4,366,241 to Tom et al.

As used herein, the term "test sample" generally refers to a material suspected of containing the analyte. The test sample may be used directly as obtained from the source or following a pretreatment to modify the character of the sample. The test sample may be derived from any biological source, such as a physiological fluid, including, blood, interstitial fluid, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, vaginal fluid, amniotic fluid or the like. The test sample may be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, and the like. Methods of treatment may involve filtration, precipitation, dilution, distillation, mixing, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples may be used such as water, food products and the like for the performance of environmental or food production assays. In addition, a solid material suspected of

containing the analyte may be used as the test sample. In some instances it may be beneficial to modify a solid test sample to form a liquid medium or to release the analyte.

Detailed Description

Reference now will be made in detail to various embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations may be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment, may be used on another embodiment to yield a still further embodiment. Thus, it is intended that the present invention covers such modifications and variations as come within the scope of the appended claims and their equivalents.

In general, the present invention is directed to a lateral flow, membrane-based assay device for detecting the presence or quantity of an analyte residing in a test sample. The device utilizes phosphorescence to detect the signals generated by excited phosphorescent labels. The labels may have a long emission lifetime so that background interference from many sources, such as scattered light and autofluorescence, is practically eliminated during detection. In addition, the phosphorescent labels may be encapsulated within particles to shield the labels from quenchers, such as oxygen or water, which might disrupt the phosphorescent signal.

Referring to Fig. 1, for instance, one embodiment of a flow-through assay device 20 that may be formed according to the present invention will now be described in more detail. As shown, the device 20 contains a porous membrane 23 optionally supported by a rigid material 21. In general, the porous membrane 23 may be made from any of a variety of materials through which the test sample is capable of passing. For example, the materials used to form the porous membrane 23 may include, but are not limited to, natural, synthetic, or naturally occurring materials that are synthetically modified, such as polysaccharides (e.g., cellulose materials such as paper and cellulose derivatives, such as cellulose acetate and nitrocellulose); polyether sulfone; polyethylene; nylon; polyvinylidene

fluoride (PVDF); polyester; polypropylene; silica; inorganic materials, such as deactivated alumina, diatomaceous earth, $MgSO_4$, or other inorganic finely divided material uniformly dispersed in a porous polymer matrix, with polymers such as vinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate copolymer; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon or rayon); porous gels, such as silica gel, agarose, dextran, and gelatin; polymeric films, such as polyacrylamide; and the like. In one particular embodiment, the porous membrane 23 is formed from nitrocellulose and/or polyether sulfone materials. It should be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, such as aliphatic carboxylic acids having from 1 to 7 carbon atoms.

The device 20 may also contain a wicking pad 28. The wicking pad 28 generally receives fluid that has migrated through the entire porous membrane 23. As is well known in the art, the wicking pad 28 may assist in promoting capillary action and fluid flow through the membrane 23.

To initiate the detection of an analyte within the test sample, a user may directly apply the test sample to a portion of the porous membrane 23 through which it may then travel. Alternatively, the test sample may first be applied to a sampling pad (not shown) that is in fluid communication with the porous membrane 23. Some suitable materials that may be used to form the sampling pad include, but are not limited to, nitrocellulose, cellulose, porous polyethylene pads, and glass fiber filter paper. If desired, the sampling pad may also contain one or more assay pretreatment reagents, either diffusively or non-diffusively attached thereto.

In the illustrated embodiment, the test sample travels from the sampling pad (not shown) to a conjugate pad 22 that is placed in communication with one end of the sampling pad. The conjugate pad 22 is formed from a material through which the test sample is capable of passing. For example, in one embodiment, the conjugate pad 22 is formed from glass fibers. Although only one conjugate pad 22 is shown, it should be understood that other conjugate pads may also be used in the present invention.

To facilitate accurate detection of the presence or absence of an analyte within the test sample, labels are applied at various locations of the device 20.

The labels may be used for both detection of the analyte and for calibration. Generally speaking, at least a portion of the labels used in the device 20 contain a phosphorescent compound. In general, such phosphorescent compounds may be phosphorescent molecules, polymers, dendrimers, particles, and so forth. In one particular embodiment, for example, the phosphorescent compound is a metal complex that includes one or more metals, such as ruthenium, osmium, rhenium, iridium, rhodium, platinum, indium, palladium, molybdenum, technetium, copper, iron, chromium, tungsten, zinc, and so forth. Especially preferred are ruthenium, rhenium, osmium, platinum, and palladium.

The metal complex may contain one or more ligands that facilitate the solubility of the complex in an aqueous or nonaqueous environments. For example, some suitable examples of ligands include, but are not limited to, pyridine; pyrazine; isonicotinamide; imidazole; bipyridine; terpyridine; phenanthroline; dipyridophenazine; porphyrin, porphine, and derivatives thereof. Such ligands may be, for instance, substituted with alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, carboxylate, carboxaldehyde, carboxamide, cyano, amino, hydroxy, imino, hydroxycarbonyl, aminocarbonyl, amidine, guanidinium, ureide, sulfur-containing groups, phosphorus containing groups, and the carboxylate ester of N-hydroxy-succinimide.

For example, porphyrins and porphine metal complexes possess pyrrole groups coupled together with methylene bridges to form cyclic structures with metal chelating inner cavities. Many of these molecules exhibit strong phosphorescence properties at room temperature in suitable solvents (e.g., water) and an oxygen-free environment. Some suitable porphyrin complexes that are capable of exhibiting phosphorescent properties include, but are not limited to, platinum (II) coproporphyrin-I and III, palladium (II) coproporphyrin, ruthenium coproporphyrin, zinc(II)-coproporphyrin-I, derivatives thereof, and so forth. Similarly, some suitable porphine complexes that are capable of exhibiting phosphorescent properties include, but not limited to, platinum(II) tetra-meso-fluorophenylporphine and palladium(II) tetra-meso-fluorophenylporphine. Still other suitable porphyrin and/or porphine complexes are described in U.S. Patent Nos. 4,614,723 to Schmidt, et al.; 5,464,741 to Hendrix; 5,518,883 to Soini; 5,922,537 to Ewart, et al.; 6,004,530 to Sagner, et al.; and 6,582,930 to

Ponomarev, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

As indicated above, bipyridine metal complexes may also be utilized in the present invention. Some examples of suitable bipyridine complexes include, but are note limited to, bis[(4,4'-carbomethoxy)-2,2'-bipyridine] 2-[3-(4-methyl-2,2'-bipyridine-4-yl)propyl]-1,3-dioxolane ruthenium (II); bis(2,2'bipyridine)[4-(butan-1-al)-4'-methyl-2,2'-bi-pyridine]ruthenium (II); bis(2,2'-bipyridine)[4-(4'-methyl-2,2'-bipyridine-4'-yl)-butyric acid] ruthenium (II); tris(2,2'bipyridine)ruthenium (II); (2,2'-bipyridine) [bis-bis(1,2-diphenylphosphino)ethylene] 2-[3-(4-methyl-2,2'-bipyridine-4'-yl)propyl]-1,3-dioxolane osmium (II); bis(2,2'-bipyridine)[4-(4'-methyl-2,2'-bipyridine)-butylamine]ruthenium (II); bis(2,2'-bipyridine)[1-bromo-4(4'-methyl-2,2'-bipyridine-4-yl)butane]ruthenium (II); bis(2,2'-bipyridine)maleimidohexanoic acid, 4-methyl-2,2'-bipyridine-4'-butylamide ruthenium (II), and so forth. Still other suitable metal complexes that may exhibit phosphorescent properties may be described in U.S. Patent Nos. 6,613,583 to Richter, et al.; 6,468,741 to Massey, et al.; 6,444,423 to Meade, et al.; 6,362,011 to Massey, et al.; 5,731,147 to Bard, et al.; and 5,591,581 to Massey, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Regardless of the type of phosphorescent label utilized, the exposure of the label to quenchers, such as oxygen or water, may result in a disruption of the phosphorescent signal. Thus, to ensure that the phosphorescent labels are capable of emitting the desired signal intensity, they are generally encapsulated within a matrix that acts as a barrier to the relevant quencher. For instance, in some embodiments, the matrix may have a low solubility in water and oxygen, and also be relatively impermeable to water and oxygen. In this manner, the phosphorescent label may be protected from emission decay that would otherwise result from exposure to oxygen or water. For example, the matrix may protect the label such that less than about 30%, in some embodiments less than about 20%, and in some embodiments, less than about 10% of the total phosphorescent signal is quenched when the detection probes are exposed to a particular quencher.

Various types of barrier matrices may be employed in the present invention to inhibit quenching of the phosphorescent compounds. For example, in some embodiments, the phosphorescent compound may be encapsulated within a

particle. Some suitable particles that may be suitable for this purpose include, but not limited to, metal oxides (e.g., silica, alumina, etc.), polymer particles, and so forth. For example, latex polymer particles may be utilized, such as those formed from polystyrene, butadiene styrenes, styreneacrylic-vinyl terpolymer, polymethylmethacrylate, polyethylmethacrylate, styrene-maleic anhydride copolymer, polyvinyl acetate, polyvinylpyridine, polydivinylbenzene, polybutyleneterephthalate, acrylonitrile, vinylchloride-acrylates, derivatives thereof, etc. Other suitable particles may be described in U.S. Patent Nos. 5,670,381 to Jou, et al. and 5,252,459 to Tarcha, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

The phosphorescent compound may be encapsulated within the particulate matrix during and/or after particle formation. In one embodiment, encapsulated latex particles are formed through well-known precipitation techniques. For example, polymer particles may be co-dissolved with the phosphorescent compound in an organic solvent. Thereafter, another solvent may then be added to co-precipitate both the phosphorescent molecules and polymer particles. Some examples of suitable solvents that may be used in such a co-precipitation process include, but are not limited to, water, acetone, acetonitrile, tetrahydrofuran, methylene chloride, cyclohexane, chloroform, ethyl ether, propyl ether, methyl acetate, methyl alcohol, ethyl alcohol, propyl alcohol, pentane, pentene, hexane, methyl ethyl ketone, and other similar solvents.

Besides precipitation, other techniques for forming encapsulated phosphorescent particles may also be used in the present invention. In one embodiment, for example, latex-based phosphorescent particles are formed using swelling techniques. Specifically, a polymer particle is swelled with a swelling agent containing one or more volatile components and phosphorescent molecules. When swollen, the phosphorescent compound may permeate through the polymer particles and become encapsulated therein. Removal of the swelling solvent results in the encapsulated particles. Emulsion polymerization may also be used to form phosphorescent particles. For example, monomers covalently tagged with a phosphorescent moiety may be co-polymerized with other monomers to form phosphorescent particles.

Regardless of the technique by which they are formed, the shape of the

encapsulated phosphorescent particles may generally vary. In one particular embodiment, for instance, the particles are spherical in shape. However, it should be understood that other shapes are also contemplated by the present invention, such as plates, rods, discs, bars, tubes, irregular shapes, etc. In addition, the size of the particles may also vary. For instance, the average size (e.g., diameter) of the particles may range from about 0.1 nanometers to about 1,000 microns, in some embodiments, from about 0.1 nanometers to about 100 microns, and in some embodiments, from about 1 nanometer to about 10 microns. For instance, "micron-scale" particles are often desired. When utilized, such "micron-scale" particles may have a average size of from about 1 micron to about 1,000 microns, in some embodiments from about 1 micron to about 100 microns, and in some embodiments, from about 1 micron to about 10 microns. Likewise, "nano-scale" particles may also be utilized. Such "nano-scale" particles may have an average size of from about 0.1 to about 10 nanometers, in some embodiments from about 0.1 to about 5 nanometers, and in some embodiments, from about 1 to about 5 nanometers.

In one embodiment of the present invention, the encapsulated phosphorescent particles form detection probes that are used to detect the presence or absence of an analyte within a test sample. In some instances, it is desired to modify the detection probes in some manner so that they are more readily able to bond to the analyte. In such instances, the detection probes may be modified with certain specific binding members to form conjugated detection probes. Specific binding members generally refer to a member of a specific binding pair, i.e., two different molecules where one of the molecules chemically and/or physically binds to the second molecule. For instance, immunoreactive specific binding members may include antigens, haptens, aptamers, antibodies, and complexes thereof, including those formed by recombinant DNA methods or peptide synthesis. An antibody may be a monoclonal or polyclonal antibody, a recombinant protein or a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific binding members are well known to those skilled in the art. Other common specific binding pairs include but are not limited to, biotin and avidin, biotin and streptavidin, antibody-binding

proteins (such as protein A or G) and antibodies, carbohydrates and lectins, complementary nucleotide sequences (including label and capture nucleic acid sequences used in DNA hybridization assays to detect a target nucleic acid sequence), complementary peptide sequences including those formed by recombinant methods, effector and receptor molecules, hormone and hormone binding protein, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, and so forth. Furthermore, specific binding pairs may include members that are analogs of the original specific binding member. For example, a derivative or fragment of the analyte, i.e., an analyte-analog, may be used so long as it has at least one epitope in common with the analyte.

The specific binding members may generally be attached to the detection probes using any of a variety of well-known techniques. For instance, covalent attachment of the specific binding members to the particles may be accomplished using carboxylic, amino, aldehyde, bromoacetyl, iodoacetyl, thiol, epoxy and other reactive or linking functional groups, as well as residual free radicals and radical cations, through which a protein coupling reaction may be accomplished. A surface functional group may also be incorporated as a functionalized co-monomer because the surface of the particle may contain a relatively high surface concentration of polar groups. In addition, although particles are often functionalized after synthesis, in certain cases, such as poly(thiophenol), the particles are capable of direct covalent linking with a protein without the need for further modification. For example, in one embodiment, the first step of conjugation is activation of carboxylic groups on the particle surface using carbodiimide. In the second step, the activated carboxylic acid groups are reacted with an amino group of an antibody to form an amide bond. The activation and/or antibody coupling may occur in a buffer, such as phosphate-buffered saline (PBS) (e.g., pH of 7.2) or 2-(N-morpholino) ethane sulfonic acid (MES) (e.g., pH of 5.3). As shown, the resulting particles may then be blocked with ethanolamine, for instance, to form the conjugated probe. Besides covalent bonding, other attachment techniques, such as physical adsorption, may also be utilized in the present invention.

In general, a variety of flow-through assay devices may be constructed according to the present invention for use in conjunction with a phosphorescence detection system. In this regard, various embodiments of the present invention will

now be described in more detail. It should be understood, however, that the embodiments discussed below are only exemplary, and that other embodiments are also contemplated by the present invention. For instance, referring again to Fig. 1, one system for detecting the presence of an analyte within a test sample is schematically illustrated. Initially, a test sample containing an analyte is applied to the sampling pad (not shown). From the sampling pad, the test sample may then travel to the conjugate pad 22, where the analyte mixes with detection probes to form analyte complexes. In one embodiment, for example, the detection probes are formed from microparticles that encapsulate a porphyrin or porphine dye, such as described above, and bound to a specific binding member for the analyte of interest. Moreover, because the conjugate pad 22 is in fluid communication with the porous membrane 23, the complexes may migrate from the conjugate pad 22 to a detection zone 31 present on the porous membrane 23.

The detection zone 31 may contain an immobilized capture reagent that is generally capable of forming a chemical or physical bond with the detection probes. In some embodiments, the capture reagent may be a biological capture reagent. Such biological capture reagents are well known in the art and may include, but are not limited to, antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, antibodies (e.g., polyclonal, monoclonal, etc.), and complexes thereof. The immobilized capture reagents serve as stationary binding sites for probe conjugate/analyte complexes. In some instances, the analytes, such as antibodies, antigens, etc., have two binding sites. Upon reaching the detection zone 31, one of these binding sites is occupied by the specific binding member of the complexed detection probes. However, the free binding site of the analyte may bind to the immobilized capture reagent. Upon being bound to the immobilized capture reagent, the complexed detection probes form a new ternary sandwich complex.

The detection zone 31 may generally provide any number of distinct detection regions so that a user may better determine the concentration of a particular analyte within a test sample. Each region may contain the same capture reagents, or may contain different capture reagents for capturing multiple analytes. For example, the detection zone 31 may include two or more distinct detection regions (e.g., lines, dots, etc.). The detection regions may be disposed in the form

of lines in a direction that is substantially perpendicular to the flow of the test sample through the assay device 20. Likewise, in some embodiments, the detection regions may be disposed in the form of lines in a direction that is substantially parallel to the flow of the test sample through the assay device.

Although the detection zone 31 may indicate the presence of an analyte, it is often difficult to determine the relative concentration of the analyte within the test sample using solely a detection zone 31. Thus, the assay device 20 may also include a calibration zone 32. In this embodiment, the calibration zone 32 is formed on the porous membrane 23 and is positioned downstream from the detection zone 31, although it may also be positioned upstream if desired. The calibration zone 32 is provided with a capture reagent that is capable of binding to probes, either uncaptured detection probes or separate calibration probes, which pass through the detection zone 31.

The capture reagents utilized in the calibration zone 32 may be the same or different than the capture reagents used in the detection zone 31. For example, biological capture reagents may be utilized. It may also be desired to utilize various non-biological reagents for the capture reagents. For instance, the capture reagents may include a polyelectrolyte that may bind to probes. The polyelectrolytes may have a net positive or negative charge, as well as a net charge that is generally neutral. For instance, some suitable examples of polyelectrolytes having a net positive charge include, but are not limited to, polylysine (commercially available from Sigma-Aldrich Chemical Co., Inc. of St. Louis, MO), polyethylenimine; epichlorohydrin-functionalized polyamines and/or polyamidoamines, such as poly(dimethylamine-co-epichlorohydrin); polydiallyldimethyl-ammonium chloride; cationic cellulose derivatives, such as cellulose copolymers or cellulose derivatives grafted with a quaternary ammonium water-soluble monomer; and so forth. In one particular embodiment, CelQuat® SC-230M or H-100 (available from National Starch & Chemical, Inc.), which are cellulosic derivatives containing a quaternary ammonium water-soluble monomer, may be utilized. Moreover, some suitable examples of polyelectrolytes having a net negative charge include, but are not limited to, polyacrylic acids, such as poly(ethylene-co-methacrylic acid, sodium salt), and so forth. It should also be understood that other polyelectrolytes may also be utilized in the present invention,

such as amphiphilic polyelectrolytes (i.e., having polar and non-polar portions). For instance, some examples of suitable amphiphilic polyelectrolytes include, but are not limited to, poly(styryl-b-N-methyl 2-vinyl pyridinium iodide) and poly(styryl-b-acrylic acid), both of which are available from Polymer Source, Inc. of Dorval, Canada.

Similar to the detection zone 31, the calibration zone 32 may provide any number of distinct calibration regions in any direction so that a user may better determine the concentration of a particular analyte within a test sample. Each region may contain the same capture reagents, or may contain different capture reagents for capturing different phosphorescent labels. The calibration regions may be pre-loaded on the porous membrane 23 with different amounts of the capture reagent so that a different signal intensity is generated by each calibration region upon migration of the probes. The overall amount of capture reagent within each calibration region may be varied by utilizing calibration regions of different sizes and/or by varying the concentration or volume of the capture reagent in each calibration region. If desired, an excess of detection probes may be employed in the assay device 20 so that each calibration region reaches its full and predetermined potential for signal intensity. That is, the amount of uncaptured detection probes that are deposited upon calibration regions are predetermined because the amount of the capture reagent employed on the calibration regions is set at a predetermined and known level. In the alternative, a predetermined amount of separate calibration probes may be used that are configured to only bind to the capture reagent at the calibration zone 32.

Once captured, the phosphorescent signal of the probes at the detection zone 31 and/or calibration zone 32 may be measured using a phosphorescence reader 50. For example, in this embodiment, the phosphorescence reader 50 is constructed to emit pulsed light simultaneously onto the detection and calibration zones 31 and 32. The reader 50 may also simultaneously receive the phosphorescent signal from the excited labels at the detection and calibration zones 31 and 32. Alternatively, the phosphorescence reader 50 may be constructed to successively emit pulsed light onto the detection zone 31 and the calibration zone 32. In addition, a separate phosphorescence reader (not shown) may also be used to measure the phosphorescent signal at the calibration zone

32.

The construction of the phosphorescence reader 50 may generally vary depending on a variety of factors, such as cost, the level of accuracy required, the nature and concentration of the analyte of interest, and so forth. In one embodiment, for example, a "time-resolved" reader may be utilized. Time-resolved detection involves exciting the phosphorescent label with one or more short pulses of light, then typically waiting a certain time (e.g., between approximately 1 to 100 microseconds) after excitation before measuring the remaining the phosphorescent signal. In this manner, any short-lived phosphorescent or fluorescent background signals and scattered excitation radiation are eliminated. This ability to eliminate much of the background signals may result in sensitivities that are 2 to 4 orders greater than conventional fluorescence or phosphorescence. Thus, time-resolved phosphorescence detection is designed to reduce background signals from the emission source or from scattering processes (resulting from scattering of the excitation radiation) by taking advantage of the phosphorescence characteristics of certain phosphorescent materials.

To function effectively, time-resolved techniques generally require a relatively long emission lifetime for the phosphorescent labels. This is desired so that the label emits its signal well after any short-lived background signals dissipate. Furthermore, a long phosphorescence lifetime makes it possible to use low-cost circuitry for time-gated phosphorescence measurements. For example, phosphorescent labels used in the present invention may have a phosphorescence lifetime of greater than about 1 microsecond, in some embodiments greater than about 10 microseconds, in some embodiments greater than about 50 microseconds, and in some embodiments, from about 100 microseconds to about 1000 microseconds. For instance, platinum (II) coproporhpyrin-I and particles encapsulated with such compounds have an emission lifetime of approximately 50 microseconds, palladium (II) coproporphyrin and particles encapsulated with such compounds have an emission lifetime of approximately 500 microseconds, and ruthenium bipyridyl complexes and particles encapsulated with such compounds have an emission lifetime of from about 1 to about 10 microseconds.

In addition, the phosphorescent label may also have a relatively large "Stokes shift." The term "Stokes shift" is generally defined as the displacement of

spectral lines or bands of luminescent radiation to a longer emission wavelength than the excitation lines or bands. A relatively large Stokes shift allows the excitation wavelength of the phosphorescent label to remain far apart from its emission wavelengths and is desirable because a large difference between excitation and emission wavelengths makes it easier to eliminate the reflected excitation radiation from the emitted signal. Further, a large Stokes shift also minimizes interference from phosphorescent molecules in the sample and/or light scattering due to proteins or colloids, which are present with some body fluids (e.g., blood). In addition, a large Stokes shift also minimizes the requirement for expensive, high-precision filters to eliminate background interference. For example, in some embodiments, the phosphorescent labels have a Stokes shift of greater than about 50 nanometers, in some embodiments greater than about 100 nanometers, and in some embodiments, from about 100 to about 350 nanometers. For instance, platinum (II) coproporhpyrin-I has a Stokes shift of approximately 260 nanometers, palladium (II) coproporphyrin has a Stokes shift of approximately 270 nanometers, and ruthenium coproporphyrin has a Stokes shift of approximately 150 nanometers.

Referring again to Fig. 1, the phosphorescence reader 50 may thus utilize time-resolved detection techniques. In such instances, the reader 50 may include one or more pulsed excitation sources and photodetectors that are in communication with each other and other optional components, such as optical filters. The use of pulsed excitation and time-gated detection, optionally combined with optical filters, allows for specific detection of the phosphorescence from only the phosphorescent label, rejecting emission from other species present in the sample that are typically shorter-lived.

For instance, referring to Fig. 2, one embodiment of an exemplary phosphorescence reader 50 is shown that includes an excitation source 52 and a detector 54. Various excitation sources 52 may be used in the present invention, including, for example, light emitting diodes (LED), flashlamps, as well as other suitable sources. Excitation illumination may also be multiplexed and/or collimated; for example, beams of various discrete frequencies from multiple coherent sources (e.g., lasers) may be collimated and multiplexed using an array of dichroic mirrors. Further, illumination may be continuous or pulsed, or may

combine continuous wave (CW) and pulsed illumination where multiple illumination beams are multiplexed (e.g., a pulsed beam is multiplexed with a CW beam), permitting signal discrimination between phosphorescence induced by the CW source and phosphorescence induced by the pulsed source. For example, gallium arsenide LED diodes (e.g., aluminum gallium arsenide red diodes, gallium phosphide green diodes, gallium arsenide phosphide green diodes, or indium gallium nitride violet/blue/ultraviolet (UV) diodes) may be used as an illumination source. One commercially available example of a suitable UV LED excitation diode suitable for use in the present invention is Model NSHU55OE (Nichia Corporation), which emits 750 to 1000 microwatts of optical power at a forward current of 10 milliamps (3.5-3.9 volts) into a beam with a full-width at half maximum of 10 degrees, a peak wavelength of 370-375 nanometers, and a spectral half-width of 12 nanometers.

Further, examples of suitable detectors 54 that may be used in the present invention include, but not limited to, photomultiplier devices; photodiodes, such as avalanche photodiodes, silicon photodiodes, etc.; high speed, linear charge-coupled devices (CCD), CID devices, or CMOS based imagers; and so forth. In one embodiment, the phosphorescent system utilizes a silicon photodiode for phosphorescent detection. Silicon photodiodes are advantageous in that they are inexpensive, sensitive, capable of high-speed operation (short risetime / high bandwidth), and easily integrated into most other semiconductor technology and monolithic circuitry. In addition, silicon photodiodes are physically small, which enables them to be readily incorporated into a system for use in membrane-based devices. If silicon photodiodes are used, then the wavelength range of the phosphorescent emission should be within their range of sensitivity, which is 400 to 1100 nanometers. Another detector option is a CdS (cadmium sulfide) photoconductive cell, which has the advantage of having a spectral sensitivity similar to that of human vision (photopic curve) that may make rejection of the reflected excitation radiation easier.

Optionally, optical filters (not shown) may be disposed adjacent to the excitation source 52 and the detector 54. The optical filters may have high transmissibility in the excitation wavelength range(s) and low transmissibility in one or more undesirable wavelength band(s) to filter out undesirable wavelengths from

the excitation source. Undesirable wavelength ranges generally include those wavelengths that produce detectable sample autofluoresence and/or are within about 25 to about 100 nanometers of excitation maxima wavelengths and thus are potential sources of background noise from scattered excitation illumination. Several examples of optical filters that may be utilized in the present invention include, but are not limited to, dyed plastic resin or gelatin filters, dichroic filters, thin multi-layer film interference filters, plastic or glass filters, epoxy or cured transparent resin filters. In one embodiment, the detector and/or excitation source may be embedded or encapsulated within the filter. Although optical filters may be utilized, one beneficial aspect of the present invention is that such filters are often not required as a result of time-resolving. Specifically, due to the delay in phosphorescence emission, emission bandwidth filters may not be required to filter out any short-lived phosphorescence emitted by the excitation source.

Referring again to Fig. 2, various timing circuitry is also used to control the pulsed excitation of the excitation source 52 and the measurement of the emitted phosphorescence. For instance, in the illustrated embodiment, a clock source 56 (e.g., a crystal oscillator) is employed to provide a controlled frequency source to other electronic components in the phosphorescence reader 50. In this particular embodiment, for instance, the oscillator 56 may generate a 20 MHz signal, which is provided to an LED driver/pulse generator 55 and to an A/D converter 64. The clock signal from oscillator 56 to A/D converter 64 controls the operating speed of A/D converter 64. It should be appreciated that a frequency divider may be utilized in such respective signal paths if the operating frequency of A/D converter 64 or if the desired frequency of the clock input to LED driver/pulse generator 55 is different than 20 MHz. Thus, it should be appreciated that the signal from oscillator 56 may be modified appropriately to provide signals of a desired frequency. In some embodiments, a signal from oscillator 56 may also be provided to microprocessor 60 to control its operating speed. Additional frequency dividers may be utilized in other signal paths in accordance with the present invention.

Microprocessor 60 provides control input to pulse generator 55 such that the 20 MHz signal from oscillator 56 is programmably adjusted to provide a desired pulse duration and repetition rate (for example, a 1 kHz source with a 50% duty

cycle). The signal from pulse generator 55 may then be provided to the excitation source 52, controlling its pulse repetition rate and duty cycle of illumination. In some embodiments, a transistor may be provided in the signal path to excitation source 52, thus providing a switching means for effecting a pulsed light signal at excitation source 52.

As described above, the pulsed light excites phosphorescent labels associated with the subject assay devices. After the desired response time (e.g., about 100 to about 200 microseconds), the detector 54 detects the phosphorescence signal emitted by the excited phosphorescent labels and generates an electric current representative thereof. This electric current may then be converted to a voltage level by a high-speed transimpedance preamplifier 78, which may be characterized by a relatively low settling time and fast recovery from saturation. The output of the preamplifier 78 may then be provided to the data input of A/D converter 64. Additional amplifier elements (such as a programmable gain amplifier) may be employed in the signal path after preamplifier 78 and before A/D converter 64 to yield a signal within an appropriate voltage range at the trailing edge of the excitation pulse for provision to the A/D converter 64. A/D converter 64 may be a high-speed converter that has a sample rate sufficient to acquire many points within the phosphorescence lifetime of the subject phosphorescent labels. The gain of the preamplifier 78 may be set such that data values drop below the maximum A/D count (e.g., 2047 for a 12-bit converter) on the trailing edge of the excitation pulse. Data within the dynamic range of A/D converter 64 would then be primarily representative of the desired phosphorescence signal. If the sample interval is short compared with the rise-time and fall-time of the excitation pulse, then the gain of preamplifier 78 may be set to ensure that signal values within the upper ½ or ¾ of the dynamic range of A/D converter 78 correspond to the trailing edge of the emission pulse.

A/D converter 64 samples the signal from preamplifier 78 and provides it to the microprocessor 60 where software instruction is configured for various processing of the digital signal. An output from the microprocessor 60 is provided to the A/D converter 64 to further control when the detected phosphorescence signal is sampled. Control signals to preamplifier 78 (not shown) and to A/D converter 64 may be continuously modified to achieve the most appropriate gain,

sampling interval, and trigger offset. It should be appreciated that although the A/D converter 64 and the microprocessor 60 are depicted as distinct components, commercially available chips that include both such components in a single module may also be utilized in the present invention. After processing, the microprocessor 60 may provide at least one output indicative of the phosphorescence levels detected by the detector 54. One such exemplary output is provided to a display 86, thus providing a user with a visual indication of the phosphorescence signal generated by the label. Display 86 may provide additional interactive features, such as a control interface to which a user may provide programmable input to microprocessor 60.

Yet another embodiment of representative specific electronic components for use in a phosphorescence reader 50 is illustrated in Fig. 3. Many of the components in Fig. 3 are analogous to those of Fig. 2 and so the same reference characters are used in such instances. For example, one difference in the reader 50 of Fig. 3 as compared to that of Fig. 2 is the generation of a gate signal at phase delay module 57. A control signal from microprocessor 60 is provided to phase delay module 57 to program the effective phase shift of a clock signal provided thereto. This shifted clock signal (also referred to as a gate signal) is then provided to a mixer 58 where such signal is multiplied by the periodic detector signal received by the detector 54 and passed through preamplifier 78. The resulting output of mixer 58 is then sent through a low-pass filter 62 before being provided to A/D converter 64. A/D converter 64 may then measure the output of low-pass filter 62 to obtain a measurement of the phosphorescence during intervals defined by the gate signal.

Still further alternative features for an exemplary phosphorescence reader embodiment 50 are illustrated in Fig. 4. For instance, a sample/hold amplifier 88 (also sometimes referred to as a track-and-hold amplifier) is shown that captures and holds a voltage input signal at specific points in time under control of an external signal. A specific example of a sample/hold amplifier for use with the present technology is a SHC5320 chip, such as those sold by Burr-Brown Corporation. The sample/hold amplifier external control signal in the embodiment of Fig. 4 is received from a delay circuit 92, which may, for instance, be digital delay circuit that derives a predetermined delay from the clock using counters,

basic logic gates, and a flip-flop circuit. Delay circuit 92 receives a clock signal from oscillator 56 and an enable signal from frequency divider 90, which simply provides a periodic signal at a reduced frequency level than that generated at oscillator 56. Delay circuit 92 may also receive a control input from microprocessor 60 to enable programmable aspects of a delay to ensure proper sampling at sample/hold amplifier 88. The delayed pulse control signal from delay circuit 92 to sample/hold amplifier 88 thus triggers acquisition of the phosphorescence signal from the detector 54 at preset time intervals after the excitation source 52 has turned off.

Regardless of the construction of the reader 50 utilized, the amount of the analyte may be ascertained by correlating the emitted phosphorescence signal, I_s, of the labels captured at the detection zone 31 to a predetermined analyte concentration. In some embodiments, the intensity signal, I_s, may also be compared with the emitted phosphorescence intensity signal, I_c, of labels captured at the calibration zone 32. The phosphorescence intensity signal I_s, may be compared to the phosphorescence intensity signal I_c. In this embodiment, the total amount of the labels at the calibration zone 32 is predetermined and known and thus may be used for calibration purposes. For example, in some embodiments (e.g., sandwich assays), the amount of analyte is directly proportional to the ratio of I_s to I_c. In other embodiments (e.g., competitive assays), the amount of analyte is inversely proportional to the ratio of I_s to I_c. Based upon the intensity range in which the detection zone 31 falls, the general concentration range for the analyte may be determined. As a result, calibration and sample testing may be conducted under approximately the same conditions at the same time, thus providing reliable quantitative or semi-quantitative results, with increased sensitivity.

If desired, the ratio of I_s to I_c may be plotted versus the analyte concentration for a range of known analyte concentrations to generate a calibration curve. To determine the quantity of analyte in an unknown test sample, the signal ratio may then be converted to analyte concentration according to the calibration curve. It should be noted that alternative mathematical relationships between I_s and I_c may be plotted versus the analyte concentration to generate the calibration curve. For example, in one embodiment, the value of $I_s /(I_s + I_c)$ may be plotted versus analyte concentration to generate the calibration curve.

As indicated above, sandwich formats, competitive formats, and so forth, may be utilized for the device 20. Sandwich assay formats typically involve mixing the test sample with antibodies to the analyte. These antibodies are mobile and linked to the label. This mixture is then contacted with a chromatographic medium containing a band or zone of immobilized antibodies to the analyte. The chromatographic medium is often in the form of a strip resembling a dipstick. When the complex of the analyte and the labeled antibody reaches the zone of the immobilized antibodies on the chromatographic medium, binding occurs and the bound labeled antibodies are localized at the zone. This indicates the presence of the analyte. This technique may be used to obtain quantitative or semi-quantitative results. Some examples of such sandwich-type assays are described by U.S. Patent Nos. 4,168,146 to Grubb, et al. and 4,366,241 to Tom, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

In a competitive assay, the probe is generally a labeled analyte or analyte-analog that competes for binding of an antibody with any unlabeled analyte present in the sample. Competitive assays are typically used for detection of analytes such as haptens, each hapten being monovalent and capable of binding only one antibody molecule. Examples of competitive immunoassay devices are described in U.S. Patent Nos. 4,235,601 to Deutsch, et al., 4,442,204 to Liotta, and 5,208,535 to Buechler, et al., which are incorporated herein in their entirety by reference thereto for all purposes. Various other device configurations and/or assay formats are also described in U.S. Patent Nos. 5,395,754 to Lambotte, et al.; 5,670,381 to Jou, et al.; and 6,194,220 to Malick, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Although various embodiments of device configurations have been described above, it should be understood, that a device of the present invention may generally have any configuration desired, and need not contain all of the components described above.

The present invention may be better understood with reference to the following examples.

EXAMPLE 1

The ability to encapsulate platinum(II)tetra-meso-fluorophenylporphine (Pt(II)-MTPFP) in carboxylated latex particles was demonstrated. Initially, 8

milligrams of a carboxylated latex particle suspension (0.3-micrometer particle size, available from Bangs Laboratories, Inc) was washed twice with ethanol and then suspended in 100 microliters of ethanol. 100 micrograms of Pt(II)MTPFP in 55 microliters of methylene chloride and 45 microliters of ethanol was added to the particle suspension. The mixture was gently shaken for 20 minutes. Then, 100 microliters of water was added. The mixture was then shaken overnight. Approximately 50 vol.% of the solvent was then removed by air stream. 1 milliliter of ethanol was added and centrifuged, after which the supernatant was discarded. The particles were further washed twice with ethanol and then twice with water. The washed particles were suspended by bath sonication in 1.5 milliliter of water for storage. The particles exhibited very strong phosphorescence at an emission wavelength of 650 nanometers when excited at 390 nanometers at ambient conditions (without removing oxygen).

EXAMPLE 2

The encapsulation of platinum(II)tetra-meso-fluorophenylporphine in polyacrylonitrile particles was demonstrated. 118.5 milligrams of polyacrylonitrile and 1.19 milligrams of platinum(II) tetra-meso-fluorophenylporphine were dissolved in 25 milliliters of DMF. 125 milliliters of water was added to the mixture under vigorous stirring. Thereafter, 1 milliliter of a saturated sodium chloride aqueous solution was added to precipitate the dispersed particles. The mixture was centrifuged for 15 minutes and then washed twice with a sodium chloride solution (5 wt.%) and three times with 50 milliliters of water. The residue was then heated for 15 minutes at 70°C and centrifuged in a phosphate buffer. This procedure is substantially identical to that described in Bioconjugated Chemistry, Kurner, 12, 883-89 (2001), with the exception that the ruthenium complexes were replaced with platinum(II) tetra-meso-fluorophenylporphine.

The resulting encapsulated particles exhibited very strong phosphorescence at an emission wavelength of 650 nanometers when excited at 390 nanometers under ambient conditions.

EXAMPLE 3

The ability to conjugate an antibody to phosphorescent particles was demonstrated. The particles were carboxylated latex phosphorescent particles having a particle size of 0.20 micrometers, 0.5% solids, and exhibiting

phosphorescence at an emission wavelength of 650 nanometers when excited at a wavelength of 390 nanometers. The particles were obtained from Molecular Probes, Inc. under the name "FluoSpheres."

Initially, 500 microliters of the particles were washed once with 1 milliliter of a carbonate buffer and twice with 2-[N-morpholino]ethanesulfonic acid (MES) buffer (pH: 6.1, 20 millimolar) using a centrifuge. The washed particles were re-suspended in 250 microliters of MES. Thereafter, 3 milligrams of carbodiimide (Polysciences, Inc.) was dissolved in 250 microliters of MES and added to the suspended particles. The mixture was allowed to react at room temperature for 30 minutes on a shaker. The activated particles were then washed twice with a borate buffer (Polysciences, Inc) and re-suspended in 250 microliters of borate buffer. 30 microliters of CRP monoclonal antibody (CRP Mab1) (3.4 milligrams per milliliter, A#5811 from Biodesign) was then added to the particle suspensions. The mixture was allowed to react at room temperature on an end-over-end shaker overnight. During the period of reaction, the suspensions were bath-sonicated twice. The particles were then collected and incubated in 250 microliters of 0.1 molar ethanolamine (Polysciences Inc.) under gentle shaking for 15 minutes. The particles were washed twice with a trizma acid/trizma base buffer (20 millimolar, pH: 7.2). The washed conjugates were suspended in 1 milliliter of the trizma acid/trizma base buffer and stored at 4°C.

EXAMPLE 4

The ability to conjugate an antibody to phosphorescent particles was demonstrated. The particles were carboxylated polyacrylonitrile phosphorescent particles having a particle size of 0.04 micrometers, 0.5% solids, and exhibiting phosphorescence at an emission wavelength of 650 nanometers when excited at a wavelength of 390 nanometers. The particles were obtained from Chromeon GmbH.

Initially, 500 microliters of the particles were suspended in 50 microliters of 2-[N-morpholino]ethanesulfonic acid (MES) buffer (0.1 molar). The particle suspension was bath-sonicated for 5 minutes. Thereafter, 6 milligrams of carbodiimide (Polysciences, Inc.) was dissolved in 50 microliters of MES and added to the suspended particles. The mixture was allowed to react at room temperature for 30 minutes on a shaker. The activated particles were then

washed twice with a borate buffer (Polysciences, Inc) and re-suspended in 250 microliters of borate buffer. 30 microliters of CRP monoclonal antibody (CRP Mab1) (3.4 milligrams per milliliter, A#5811 from Biodesign) was then added to the particle suspensions. The mixture was allowed to react at room temperature on an end-over-end shaker overnight. During the period of reaction, the suspensions were bath-sonicated twice. The particles were then collected and incubated in 250 microliters of 0.1 molar ethanolamine (Polysciences, Inc.) under gentle shaking for 15 minutes. The particles were washed twice with a trizma acid/trizma base buffer (20 millimolar, pH: 7.2). The washed conjugates were suspended in 1 milliliter of the trizma acid/trizma base buffer and stored at 4°C.

EXAMPLE 5

The ability to form a lateral flow assay device with using phosphorescent particles was demonstrated. A nitrocellulose porous membrane (HF 120 from Millipore, Inc.) having a length of approximately 30 centimeters was laminated onto supporting cards. Goldline™ (a polylysine solution obtained from British Biocell International) was stripped onto the membrane to form a calibration line. In addition, monoclonal antibody for C-reactive protein (Mab2) (A#5804, available from Biodesign, concentration of 1 milligram per milliliter) was immobilized on the porous membrane to form a detection line. The membrane samples were then dried for 1 hour at a temperature of 37°C. A cellulosic fiber wicking pad (Millipore, Inc. Co.) was attached to one end of the membrane and cut into 4-millimeter half strips.

200 microliters of the conjugated phosphorescent particles of Example 3 (concentration of 2.5 milligrams per milliliter in a trizma acid/trizma base buffer with 1 milligram per milliliter BSA) was applied with 200 microliters of Tween 20 (2%, available from Aldrich) and 200 microliters of sucrose in water (10%). The mixture was bath-sonicated for 20 minutes. The suspension was then loaded onto a 10-centimeter long glass fiber conjugate pad (Millipore Co.). The glass fiber pad was then dried at 37°C for 2 hours. Thereafter, 600 microliters of Tween 20 (0.5%) was loaded onto a 10-centimeter long glass fiber sample pad (Millipore Co.) and dried at 37°C for 2 hours. A cellulose wicking pad (Millipore Co.), the sample pad, and conjugate pad were then laminated onto the porous membrane. The laminated full card was then cut into a 4-millimeter wide lateral flow assay device.

EXAMPLE 6

The ability to form a lateral flow assay device with using phosphorescent particles was demonstrated. A nitrocellulose porous membrane (HF 120 from Millipore, Inc.) having a length of approximately 30 centimeters was laminated onto supporting cards. Goldline™ (a polylysine solution obtained from British Biocell International) was stripped onto the membrane to form a calibration line. In addition, monoclonal antibody for C-reactive protein (Mab2) (A#5804, available from Biodesign, concentration of 1 milligram per milliliter) was immobilized on the porous membrane to form a detection line. The membrane samples were then dried for 1 hour at a temperature of 37°C. A cellulosic fiber wicking pad (Millipore, Inc. Co.) was attached to one end of the membrane and cut into 4-millimeter half strips.

The half stick strips were put into a control and test microwell. The control microwell contained 200 microliters of the conjugated phosphorescent particles of Example 4 (concentration of 2.5 milligrams per milliliter in a trizma acid/trizma base buffer), 15 microliters of Tween 20 (2%, available from Aldrich), and 20 microliters of the trizma acid/trizma base buffer. The test microwell contained 200 microliters of the conjugated phosphorescent particles of Example 4 (concentration of 2.5 milligrams per milliliter in a trizma acid/trizma base buffer), 15 microliters of Tween 20 (2%, available from Aldrich), 15 microliters of the trizma acid/trizma base buffer, and C-reactive protein (2 micrograms per milliliter, available from Biodesign).

A half-stick was inserted into each microwell and allowed to develop for 20 minutes. When the assay was finished, the half stick was taken out mounted onto the sample holder of a Fluorolog III Spectrofluorometer (available from SA Instruments, Inc.) using tape. The detection line fit into a rectangular hole in the holder so that the excitation beam would shine directly on the detection line while the rest of the device was remained blocked from the excitation beam. Time-resolved phosphorescence techniques were used. Specifically, the following experiment parameters were used: (1) the angle of the excitation beam to the surface normal of the devices was 70°C; the detection mode was front face; the slit width was 5 nanometer; (4) the number of scan was 1; (5) the excitation wavelength was 390 nanometers; (6) the emission wavelength was from 600 to 700 nanometers; (7) the sample window was 2 milliseconds (ms); (8) the initial

delay was 0.02 ms; (9) the time-per-flash was 50 ms; and (10) the number of flashes was 10.

For the control, the measured phosphorescent intensity was 1.82K (K=1000), while the intensity for the test sample was 8.63K. The phosphorescent intensity was directly related to the quantity of the sandwich complex for the antigen, and therefore directly related to the concentration of the CRP antigen.

EXAMPLE 7

The ability to detect the presence of an analyte using a lateral flow assay device was demonstrated. A nitrocellulose porous membrane (HF 120 from Millipore, Inc.) having a length of approximately 30 centimeters was laminated onto supporting cards. Goldline™ (a polylysine solution obtained from British Biocell International) was stripped onto the membrane to form a calibration line. In addition, monoclonal antibody for C-reactive protein (Mab2) (A#5804, available from Biodesign, concentration of 1 milligram per milliliter) was immobilized on the porous membrane to form a detection line. The membrane samples were then dried for 1 hour at a temperature of 37°C. A cellulosic fiber wicking pad (Millipore, Inc. Co.) was attached to one end of the membrane and cut into 4-millimeter half strips. 200 microliters of the conjugated phosphorescent particles of Example 3 (concentration of 2.5 milligrams per milliliter in a trizma acid/trizma base buffer with 1 milligram per milliliter BSA) was applied with 200 microliters of Tween 20 (2%, available from Aldrich) and 200 microliters of sucrose in water (10%). The mixture was bath-sonicated for 20 minutes. The suspension was then loaded onto a 10-centimeter long glass fiber conjugate pad (Millipore Co.). The glass fiber pad was then dried at 37°C for 2 hours. Thereafter, 600 microliters of Tween 20 (0.5%) was loaded onto a 10-centimeter long glass fiber sample pad (Millipore Co.) and dried at 37°C for 2 hours. A cellulose wicking pad (Millipore Co.), the sample pad, and conjugate pad were then laminated onto the porous membrane. The laminated full card was then cut into a 4-millimeter wide lateral flow assay device.

Six (6) of the assay devices were tested. 50 microliters of a mixture of hepes buffer (N-[2-hydroxyethyl]piperazine-N'-(2-ethanesulfonic acid) from Sigma, pH: 7.4) and C-reactive protein were applied to the sample pad. Different concentrations of CRP were tested, i.e., 0, 20, 50, 100, 200 and 1000 nanograms per milliliter. The devices were allowed to develop for 30 minutes. The

phosphorescent intensity was measured as described above in Example 6. The intensity at the detection line for CRP concentrations of 0, 20, 50, 100, 200 and 1000 nanograms per milliliter was determined to be 7.21K, 6.03K, 9.31K, 8.05K, 13.7K, 19.8K, respectively. Thus, as shown, the phosphorescent intensity was directly related to the CRP antigen.

While the invention has been described in detail with respect to the specific embodiments thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing, may readily conceive of alterations to, variations of, and equivalents to these embodiments. Accordingly, the scope of the present invention should be assessed as that of the appended claims and any equivalents thereto.